8. C. L. Ehlers *et al.*, *ibid.* **278**, 332 (1983).
9. D. R. Britton, G. F. Koob, J. Rivier, W. Vale, *Life Sci.* **31**, 363 (1982); J. E. Morley and A. S. Levine, *ibid.*, p. 1459; D. R. Britton, D. K. Hoffman, K. Lederis, J. Rivier, *Brain Res.*, in press; A. S. Levine, B. Rogers, J. Kneip, M. Grace, J. E. Morley, *Neuropharmacology* **22**, 337 (1983).
10. R. E. Sutton, G. F. Koob, M. Le Moal, J. Rivier, W. Vale, *Nature (London)* **297**, 331 (1982).
11. A. J. Fischman and R. L. Moldow, *Peptides* **1**, 149 (1982); K. Hashimoto *et al.*, *Life Sci.* **32**, 1001 (1983); J. Cote, G. Lefevre, F. Labrie, N. Barden, *Regul. Peptides* **5**, 189 (1983); M. Palkovits, M. J. Brownstein, W. Vale, *Neuroendocrinology* **37**, 302 (1983).
12. J. A. Olschowka, T. L. O'Donohue, G. P. Mueller, D. M. Jacobowitz, *Peptides* **3**, 995 (1982); I. Merchenthaler, S. Vigh, P. Petrusz, A. V. Schally, *Am. J. Anat.* **165**, 385 (1982); L. W. Swanson, P. E. Sawchenko, J. Rivier, W. W. Vale, *Neuroendocrinology* **36**, 165 (1983); S. Cummings, R. Elde, J. Ells, A. Lindall, *J. Neurosci.* **3**, 1355 (1983); W. K. Paull and F. P. Gibbs, *Histochemistry* **78**, 303 (1983).
13. D. J. S. Sirinathsinghji, L. H. Rees, J. Rivier, W. Vale, *Nature (London)* **305**, 232 (1983).
14. E. B. De Souza, M. H. Perrin, J. Rivier, W. W. Vale, M. J. Kuhar, *Brain Res.* **296**, 202 (1984).
15. E. B. De Souza *et al.*, in preparation.
16. J. R. Unnerstall, R. D. Mashall, E. B. De Souza, M. J. Kuhar, in preparation; J. R. Unnerstall, D. L. Niehoff, M. J. Kuhar, M. J. Palacios, *J. Neurosci. Methods* **6**, 59 (1982).
17. P. C. Wynn *et al.*, *Biochem. Biophys. Res. Commun.* **110**, 602 (1983).
18. R. A. Peterfreund and W. W. Vale, *Endocrinology* **112**, 1275 (1983).
19. J. M. Palacios, D. L. Niehoff, M. J. Kuhar, *Neurosci. Lett.* **24**, 111 (1981).
20. W. S. Young III and M. J. Kuhar, *Brain Res.* **179**, 255 (1979).
21. We thank N. Taylor, S. Goehring, R. Proctor, and Y. Haas for technical assistance and D. Weimer for manuscript preparation. This study was supported by PHS grants MH25951 and MH00053. Research at the Peptide Biology Laboratory was supported in part by NIH grants AM26741 and HD13527 and was conducted in part by the Clayton Foundation for Research, California Division. M.H.P. and W.W.V. are Clayton Foundation investigators.
* To whom correspondence should be addressed.

6 March 1984; accepted 4 April 1984

Dopaminergic Neurotoxicity of 1-Methyl-4-Phenyl-1,2,5,6-Tetrahydropyridine in Mice

Abstract. *1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is known to cause an irreversible destruction of the dopaminergic nigrostriatal pathway and symptoms of parkinsonism in humans and in monkeys. However, MPTP has been reported to act only minimally or not at all in several other animal species. When MPTP (30 milligrams per kilogram of body weight) was administered parenterally to mice, a decrease in concentrations of neostriatal dopamine and its metabolites, a decrease in the capacity of neostriatal synaptosomal preparations to accumulate [^{3}H]dopamine, and a disappearance of nerve cells in the zona compacta of the substantia nigra were observed. In contrast, MPTP administration had no effect on neostriatal concentrations of serotonin and its metabolites. MPTP administration thus results in biochemical and histological changes in mice similar to those reported in humans and monkeys and similar to those seen in Parkinson's disease in humans. The mouse should prove to be a useful small animal with which to study the mode of action of MPTP.*

1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) is a commercially available compound that can be formed as a by-product in high yield in the synthesis of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), a potent analgesic structurally similar to two other widely used analgesics, meperidine (Demerol) and alphaprodine (Nisentil). The inadvertent ingestion of small amounts of MPTP, mixed with varying amounts of MPPP and perhaps other agents, has caused an irreversible parkinsonism in several young individuals, who were most likely attempting to simulate the actions of heroin with MPPP (*1*). Also, intravenous injection of MPTP alone to monkeys has produced symptoms and pathology consistent with parkinsonism, including a severe dopamine depletion and a highly selective degeneration of the nerve cells of the substantia nigra (*2*). Therefore, the agent responsible for the parkinsonism observed in the young drug addicts was MPTP or a metabolite.

The discovery that a simple substance of this nature administered systemically can selectively destroy a specific neuronal system and closely reproduce the pathology of Parkinson's disease has implications for the etiology of the naturally occurring human disease. It suggests that a similar neurotoxin, exogenous or endogenous, may be involved in the pathogenesis of the disease. Such a suggestion is particularly timely in view of the recent findings of a low concordance rate for Parkinson's disease among monozygotic twins (*3*).

Surprisingly, MPTP was reported to have no neurotoxic effect in several other animal species, including rats and cats (*4*). We now report that MPTP administration to mice (two to ten daily injections) resulted in a decrease in the dopamine content of neostriatal brain tissue, inability of this tissue to accumulate [^{3}H]dopamine, and a severe loss of nerve cells in the zona compacta of the substantia nigra.

Male Swiss-Webster mice weighing between 25 and 35 g were injected intraperitoneally with MPTP dissolved in distilled water (*p*H adjusted to 8.5 with dilute hydrochloric acid). The dose was 30 mg (0.17 mmole) per kilogram of body weight, and the injection volume was 1.0 ml per 100 g of body weight. Control animals received either vehicle injection or no injection at all. Since dopamine concentrations in these animals did not differ, both groups were combined and considered as controls. Some animals received multiple MPTP injections 24 hours apart. Concentrations of dopamine in the mouse neostriatum were assayed at various times after the last injection. Each animal was stunned by a blow to the head, the brains were removed, and the neostriata were rapidly dissected from the rest of the brain. The neostriata were then weighed and homogenized in 0.1*M* perchloric acid containing dihydroxybenzylamine (DHBA) as an internal standard and centrifuged at 27,000*g* for 15 minutes. The supernatant, usually 20 μl in volume (equivalent to 0.2 mg of original tissue), was used for assays of dopamine, serotonin, and their metabolites (*5*).

An LC-304T liquid chromatograph (Bioanalytical Systems) connected to a dual pen recorder (Kipp and Zonen) was used for all assays. The operating potential was 750 mV, the temperature was 25°C, and full scale on the detector was usually set to 2.0 nA. A Biophase ODS 5-μm column (Bioanalytical Systems) was used for all separations. The flow rate of the mobile phase was 1.5 ml per minute. The mobile phase was made up as follows: 35 ml of acetonitrile was added to 965 ml of 0.15*M* monochloroacetate buffer (*p*H 3.0) containing 193 mg of sodium octyl sulfate. This mixture was filtered and degassed, tetrahydrofuran (18 ml) was carefully added, and the mobile phase was sealed until use.

Data were calculated from standard curves made on the same day on the basis of five to six data points in the same concentration range as that present in the tissue. Concentrations of dopamine and its major metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were calculated on the basis of the original tissue weight, and the results were expressed in micrograms per gram of tissue ± standard error of the mean (S.E.M.).

In some experiments, the neostriata from control and MPTP-treated mice were homogenized in 0.3*M* sucrose. To a sample of this homogenate, an equal volume 0.2*M* perchloric acid containing DHBA was added. This mixture was then centrifuged, and levels of dopamine and its metabolites were assayed as de-

Table 1. Neostriatal concentrations of dopamine and its metabolites after injections of MPTP (30 mg per kilogram of body weight) to mice. Animals were given the indicated number of injections, and assays were performed between 24 and 432 hours after the time of the last injection. Data represent the mean ± S.E.M. for the number of mice indicated. All values for MPTP-treated mice, except those noted, differ significantly from control ($P < 0.001$). Statistical analysis was by Student's t-test.

Group	Injections (number)	Assay time (hours)	n	Neostriatal concentration (microgram per gram of tissue)		
				Dopamine	DOPAC	HVA
Control			33	12.7 ± 0.2	4.3 ± 0.2	1.9 ± 0.1
MPTP	1	24	7	10.9 ± 0.8*	3.2 ± 0.4†	1.7 ± 0.2‡
MPTP	2	24	10	6.6 ± 0.4	2.1 ± 0.2	1.4 ± 0.1§
MPTP	3	24	14	4.7 ± 0.5	2.1 ± 0.2	1.2 ± 0.1
MPTP	3	72	13	5.5 ± 0.4	2.1 ± 0.3	1.0 ± 0.1
MPTP	5	72	7	4.5 ± 0.6	2.1 ± 0.1	1.2 ± 0.2§
MPTP	5	240	3	5.1 ± 0.7	1.2 ± 0.3	1.0 ± 0.2
MPTP	10	288	7	2.6 ± 0.4	1.0 ± 0.2	0.9 ± 0.1
MPTP	10	432	6	3.3 ± 0.3	1.2 ± 0.2	1.1 ± 0.1

*$P < 0.05$ compared to control. †$P < 0.025$ compared to control. ‡No significant difference from control. §$P < 0.005$ compared to control.

scribed above. In the remaining 0.3M sucrose homogenate, the uptake of [^{3}H]dopamine was measured in a synaptosomal preparation (*6*). All data were calculated as disintegrations per minute of [^{3}H]dopamine accumulated per milligram of tissue used (± S.E.M.), and the data were expressed as a percent of control.

One injection of MPTP had a slight but significant effect on the neostriatal concentrations of dopamine and DOPAC (Table 1). After two and three injections of MPTP (assays at 24 hours), pronounced and significant decreases in concentrations of dopamine, DOPAC, and HVA occurred. For example, after two injections of MPTP, the mean dopamine concentration dropped to 6.6 μg/g and, after three injections, to 4.7 μg/g. When the mice were given three injections of MPTP and killed 72 hours after the last injection, large decreases in the level of dopamine and its metabolites were still observed compared to the control values (Table 1). Mice given five injections of MPTP, with assays performed 72 hours after the last injection of MPTP, had a mean dopamine concentration of 4.5 μg/g (65 percent loss) and a 67 percent loss in their capacity to accumulate [^{3}H]dopamine compared to control. In these experiments, neostriatal synaptosomal preparations from control mice accumulated 295,155 ± 14,532 dis/min of [^{3}H]dopamine per 5 mg of tissue (100 ± 4 percent, n = 7), while neostriatal synaptosomal preparations from MPTP-treated mice accumulated only 96,606 ± 12,106 dis/min of [^{3}H]dopamine per 5 mg of tissue (33 ± 4 percent of control, n = 7). Thus the decrease in [^{3}H]dopamine uptake and in the endogenous dopamine concentrations caused by MPTP were identical.

Mice given five or ten injections of MPTP, with assays performed between 240 and 432 hours after the last injection, also had pronounced decreases in concentrations of dopamine, DOPAC, and HVA (Table 1). Thus, no apparent recovery occurred for as long as 432 hours (18 days) after the last MPTP injection. In these experiments, MPTP had no effect on neostriatal concentrations of serotonin or its metabolite, 5-hydroxyindoleacetic acid. Moreover, no consistent effect of MPTP on the concentrations of dopamine or its metabolites was observed in either the nucleus accumbens or in the hypothalamus. For example, mice given ten injections of MPTP, with assays performed 120 hours after the last injection, had a dopamine concentration of 5.2 ± 0.7 μg/g in the nucleus accumbens and 0.7 ± 0.1 μg/g in the hypothalamus. Control values were 6.0 ± 1.5 and 0.6 ± 0.1 μg/g in the nucleus accumbens and hypothalamus, respectively (n = 5 in each group). Similarly, no effects of MPTP on concentrations of DOPAC or of HVA were observed in the nucleus accumbens and hypothalamus. Thus, these actions of MPTP are specific in nature, as has been reported for the monkey (*4*).

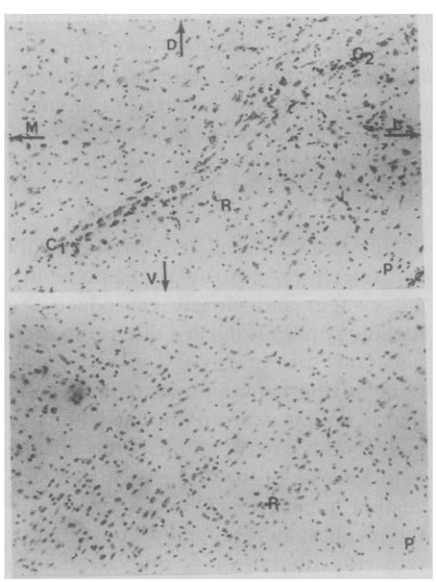

Fig. 1 (top). (Control) Coronal section through the superior colliculus at the level of the medial geniculate body. C_1 and C_2 indicate the extent of the pars compacta of the substantia nigra as it courses diagonally across the photograph dorsal to the pars reticulata (R) and cerebral peduncle (P). The M arrow points to medial direction; L arrow, lateral; D arrow, dorsal; V arrow, ventral. Fig. 2 (bottom). MPTP-treated section 15 days after ten daily injections at the same level of the superior colliculus and orientation as above. Neurons of the pars compacta of the substantia nigra are depleted in number. R and P as in Fig. 1 (×60). Similar losses of neurons were seen in several other MPTP-treated mice.

Histopathological studies of control and MPTP-treated mice were performed on littermates of animals whose content of dopamine was biochemically assessed to be reduced by MPTP administration by at least 65 to 80 percent. After perfusion of the mice with Formalin, the brains were excised. Serial paraffin sections (10 μm in thickness) through the superior colliculus containing the substantia nigra were stained with cresyl violet. Tissue from the MPTP-treated mice showed a marked decrease in cell number in the zona compacta for the entire rostro-caudal extent of the nigra (Figs. 1 and 2).

The decrease in neostriatal concentrations of dopamine and its major metabolites, the pronounced decrease in the capacity of neostriatal synaptosomes to accumulate [^{3}H]dopamine, and the marked loss of nerve cells in the zona compacta of the substantia nigra taken together show that MPTP causes degeneration of the nigrostriatal neuronal pathway in male Swiss-Webster mice. Thus, MPTP exerts its selective neurotoxic effects in the mouse as well as in humans and monkeys. The MPTP-treated mouse and monkey both exhibit this loss of nerve cells in the substantia nigra as well as the marked reduction in neostriatal concentrations of dopamine and its metabolites, two of the most important pathological changes observed in human Parkinson patients.

The advantages of working with a small animal such as the mouse should facilitate research on the metabolism and mechanism of action of MPTP. The MPTP-treated mouse may prove to be a useful model of Parkinson's disease and of related neuronal system degenerations. We have found the C57 black mouse to be considerably more sensitive

than the Swiss-Webster mouse to the neurotoxic effects of MPTP. Between seven and ten injections of MPTP in the C57 black mouse consistently led to about a 90 percent loss of neostriatal dopamine. These MPTP-treated mice exhibited postural abnormalities and had a marked reduction in their locomotor activity. In contrast, the Swiss-Webster mouse appears relatively normal even with losses of neostriatal dopamine greater than 80 percent.

In guinea pigs, 21 daily injections were required to induce a 50 percent decrease in dopamine concentrations (*4*). The differences in susceptibility to MPTP in different animal species are provocative and warrant further study. Differences in transport, absorption, metabolism, selective localization, or capacity to detoxify the neurotoxic species (either MPTP or some reactive metabolite or by-product) may be expected to account for these observations. Clarification of these differences may be important to an understanding of the mode of action of MPTP (*7*) and may yield clues to the etiology of Parkinson's disease in humans (*8*).

RICHARD E. HEIKKILA
Department of Neurology, University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway 08854

ARTHUR HESS
Department of Anatomy, University of Medicine and Dentistry of New Jersey, Rutgers Medical School

ROGER C. DUVOISIN
Department of Neurology, University of Medicine and Dentistry of New Jersey, Rutgers Medical School

References and Notes

1. G. C. Davis *et al.*, *Psychiatry Res.* **1**, 249 (1979); J. W. Langston, P. Ballard, J. W. Tetrud, I. Irwin, *Science* **219**, 979 (1983).
2. R. S. Burns, C. C. Chiueh, S. P. Markey, M. H. Ebert, D. M. Jacobowitz, I. J. Kopin, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4546 (1983).
3. C. Ward *et al.*, *Neurology* **33**, 815 (1983).
4. C. C. Chiueh *et al.*, *Pharmacologist* **25**, 131 (1983).
5. G. S. Mayer and R. E. Shoup, *J. Chromatogr.* **255**, 533 (1983).
6. O. S. Gershanik, R. E. Heikkila, R. C. Duvoisin, *Brain Res.* **174**, 345 (1979).
7. In other experiments to define important structural aspects of MPTP, we gave 4-phenyl-1,2,5,6-tetrahydropyridine (PTP) and 1-methyl-1,2,5,6-tetrahydropyridine (MTP) to animals at a dose of 0.17 mmole per kilogram of body weight (equivalent to the 30-mg dose of MPTP). At this dose of each compound administered in injection sequences as described (see Table 1), we found no significant effects of PTP or MTP on dopamine concentrations. From these experiments we conclude that MPTP is considerably more potent than either PTP or MTP and that both the 1-methyl group and the 4-phenyl group are important in its effects.
8. Supported in part by grants from the U.S. Public Health Service and from the United Parkinson Foundation. We thank L. Manzino and F. S. Cabbat for assistance and W. J. Nicklas for helpful discussions.

28 November 1983; accepted 2 May 1984

Identification of Common Interneurons Mediating Pre- and Postsynaptic Inhibition in the Cat Spinal Cord

Abstract. *The spike-triggered averaging of dorsal and ventral root potentials permits the identification of two populations of interneurons in the intermediate nucleus of the cat spinal cord. One produced negative dorsal root potentials and inhibitory ventral root potentials, in some cases with monosynaptic latencies, suggesting that they mediate presynaptic inhibition of group I afferent fibers from muscles and postsynaptic inhibition of motoneurons. The other population mediated only nonreciprocal postsynaptic inhibition of motoneurons.*

Primary afferent depolarization (PAD) is generally considered to cause presynaptic inhibition, but its nature and the mechanisms producing it remain uncertain (*1, 2*). This lack, together with the limited information available concerning the identity of the interneurons mediating the PAD, their connections, and the inputs received by them (*3–5*), has restricted our understanding of the functional role played by presynaptic inhibition in the vertebrate spinal cord.

Recent studies aimed at determining the sites of action of cutaneous and descending fibers on the pathways producing PAD of group I afferent fibers (*5*) have suggested that different last-order interneurons in the intermediate nucleus mediate the PAD of group Ia fibers (from muscle spindles) and Ib fibers (from tendon organs) (*5, 6*), and have provided criteria that, together with those already available (*1, 7, 8*), can be used for their characterization (*5*). Final identification of these interneurons, however, requires the demonstration of functional connections between them and their target afferent fibers, a condition not met in previous studies (*3, 4*). We have now used the spike-triggered averaging technique (*9–12*) to disclose the dorsal root potentials (DRP's) produced by single interneurons in the intermediate nucleus. These potentials are usually taken as indicators of synaptic actions exerted on afferent fibers (*1, 3, 6, 8*). Simultaneous spike-triggered averaging of ventral root potentials (VRP's) recorded with the sucrose gap technique (*9, 11*) has, in addition, allowed us to test the extent to which the PAD-producing interneurons form part of private pathways and determine whether they are also shared by other reflex pathways producing postsynaptic actions on motoneurons.

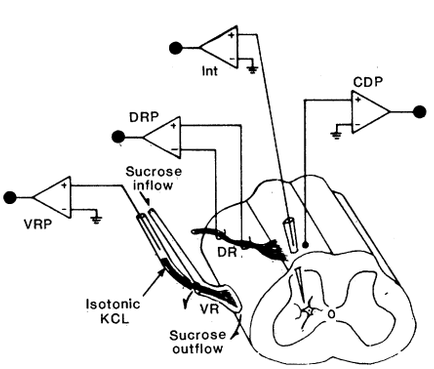

Fig. 1. Diagram of the method. Abbreviations: *Int*, interneuronal activity; *CDP*, cord dorsum potentials; *DR*, dorsal root; and *VR*, ventral root.

Cats were anesthetized with pentobarbital (35 mg per kilogram of body weight) supplemented (10 mg hourly) to maintain a deep level of anesthesia, paralyzed with Pavulon (pancuronium bromide), and connected to a respirator. The posterior biceps and semitendinosus, sural, gastrocnemius-soleus, deep and superficial peroneus nerves were dissected and prepared for stimulation. The lumbosacral spinal cord was exposed, and ventral roots L7 and S1 were dissected and sectioned distally. A small S1 dorsal rootlet was used to record the DRP's, and synaptic potentials of motoneurons were recorded from S1 ventral rootlets by means of the sucrose gap technique (*9, 11*) (Fig. 1). Action potentials of interneurons responding mono-, di-, or trisynaptically to group I gastrocnemius-soleus or posterior biceps and semitendinosus stimulation (*5, 6*) or polysynaptically to cutaneous nerve stimulation were extracellularly recorded by means of glass micropipettes (2- to 4-μm tip diameter) filled either with sodium chloride or with sodium glutamate (3*M*), the latter to increase the firing frequency of the neurons (*9, 10*). The DRP's and VRP's were recorded through low-noise differential amplifiers (band-pass filters, 0.1 Hz to 10 kHz). Cord dorsum potentials, DRP's, VRP's, and interneuronal activity were stored on analog tape. Averaging was performed on-line and off-line with a Nicolet 1170 computer.

We have analyzed the activation patterns and the connections with afferents and motoneurons of 130 interneurons located in the intermediate nucleus (laminae V and VI of Rexed). Eleven interneurons produced inhibitory VRP's (iVRP's) without any associated DRP's (Fig. 2, B and C) lasting 68.5 ± 25 msec (mean ± standard deviation). The